

## Development of Multichannel Devices with an Array of Electrospray Tips for High-Throughput Mass Spectrometry

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The basic principles of multichannel devices with an array of electrospray tips for high-throughput infusion electrospray ionization mass spectrometry (ESI-MS) have been developed. The prototype plastic devices were fabricated by casting from a solvent-resistant resin. The sample wells on the device were arranged in the format of the standard 96-microtiter well plate, with each sample well connected to an independent electrospray exit port via a microchannel with imbedded electrode. A second plastic plate with distribution microchannels was employed as a cover plate and pressure distributor. Nitrogen gas was used to pressurize individual wells for transport of sample into the electrospray exit port. The design of independent microchannels and electrospray exit ports allowed very high throughput and duty cycle, as well as elimination of any potential sample carryover. The device was placed on a computer-controlled translation stage for precise positioning of the electrospray exit ports in front of the mass spectrometer sampling orifice. High-throughput ESI-MS was demonstrated by analyzing 96 peptide samples in 480 s, corresponding to a potential throughput of 720 samples/h. As a model application, the device was used for the MS determination of inhibition constants of several inhibitors of HIV-1 protease.

The acceleration of drug discovery in recent years has presented significant analytical challenges. The number of compounds to be analyzed has increased dramatically since the introduction of combinatorial chemistry with automated parallel synthesis.<sup>1–4</sup> High-throughput analytical techniques have become critical for determining the identity and purity of synthesized substances,<sup>5</sup> as well as for clinical screening,<sup>6</sup> pharmacokinetics,<sup>7</sup> and proteome-related research.<sup>8</sup>

Most of the current protocols for high-throughput analysis are based on 96 (or larger) microtiter well plate technology where a large number of samples can be processed in parallel. Robotic work stations for such formats are used for sample dispensing and handling. Optical absorbance or fluorescence readers monitor the respective sample/reaction properties directly in the arrays,<sup>9</sup> or flow injection analysis (FIA) systems deliver samples to an external detector for measurement.<sup>10</sup>

Mass spectrometry has become an indispensable tool for pharmaceutical research because of its sensitivity, capability of sample identification, structure elucidation, and quantitation. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the frequently used sample ionization techniques for automated high-throughput MS analysis, often coupled on-line with liquid chromatography (LC) or capillary electrophoresis (CE).<sup>11–14</sup> Nevertheless, a significant portion of ESI-MS applications are also performed in the direct infusion mode.<sup>15</sup> Typically, infusion ESI-MS is carried out with a FIA system equipped with an autosampler. Since every sample flows through the same conduit from the sampling probe through the injection valve to the ESI tip, the sampling probe must be carefully washed and the flow conduit appropriately flushed to minimize sample cross-contamination.<sup>16</sup> Thus, useful mass spectrometric information can be observed only during a fraction of the total analysis time, leading to a low duty cycle.

Recently, microfluidic devices or microchips fabricated on glass, quartz, or plastic substrates have emerged as a means of handling small quantities of samples and achieving high analysis

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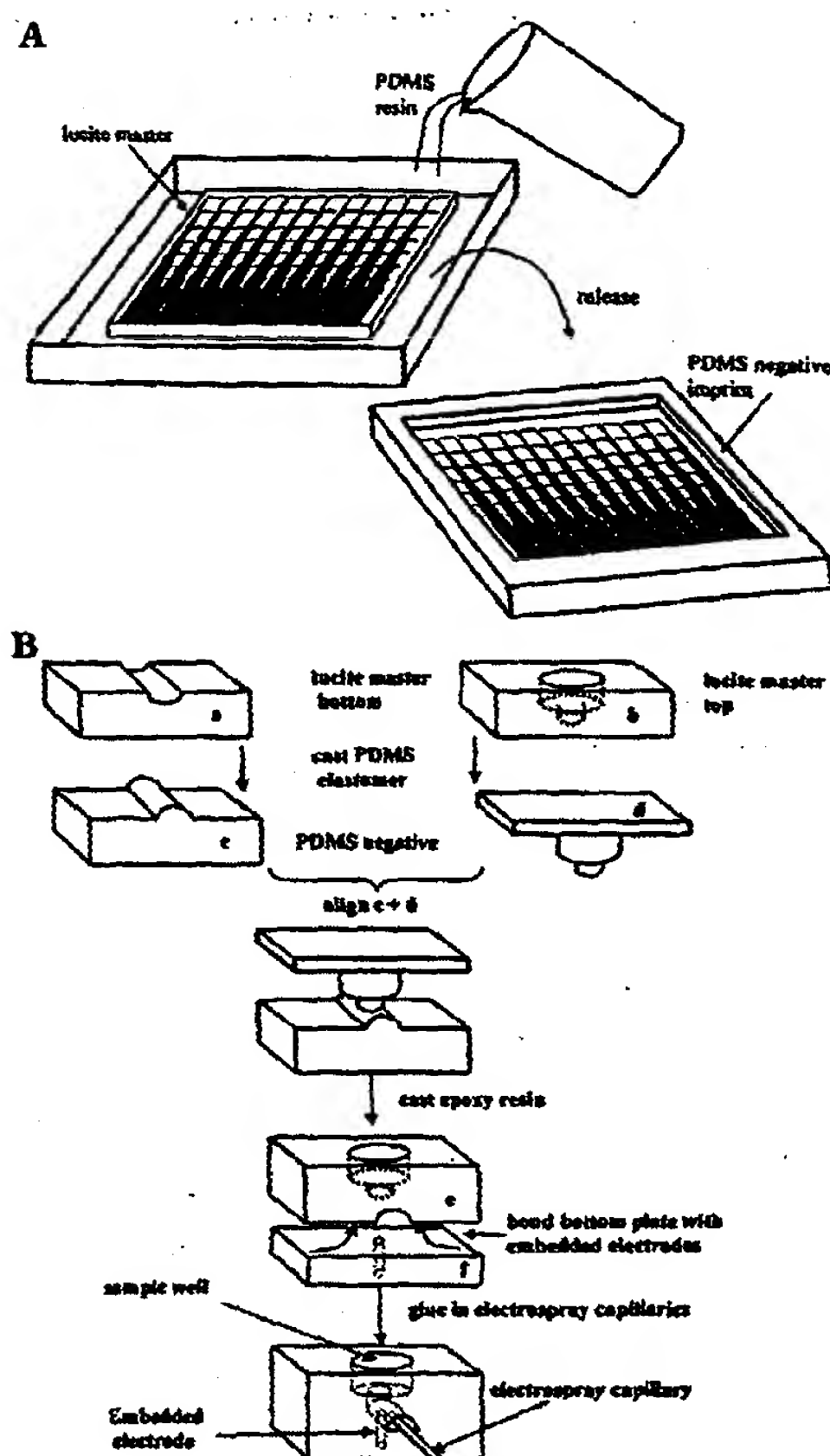
speed.<sup>17,18</sup> In previous work, we,<sup>19</sup> as well as others,<sup>20–22</sup> described the use of such microfluidic devices for generation of electrospray with MS detection. Single- and multiple-channel glass chips were successfully interfaced to ESI-MS for sample infusion,<sup>23</sup> as well as for CE separation.<sup>24–28</sup>

Considering the wide acceptance of the microtiter well plate format in automated analysis and the potentially low cost of plastic devices, a disposable device equipped with an independent electrospray exit port for each sample well represents an attractive alternative to FIA. A device with sample reservoirs positioned in the format of a standard microtiter well plate could be used as the final receiving plate in a parallel sample-processing scheme, such as selective enrichment, affinity capture, and desalting. The advantage of such a device compared to the standard FIA method would be significantly simplified instrumentation, fast switching times for analysis of consecutive samples (high duty cycle), and elimination of sample cross-contamination. Especially, the latter advantage leads to a significantly decreased number of runs required to validate that sample cross-contamination did not occur.

In this work, we have developed a prototype plastic multi-sprayer device interfaced to ESI-MS. Each of the sample wells was connected by an independent microchannel to a separate sprayer. All samples loaded onto the well plate could be analyzed in rapid sequence without need for injection or washing. When coupled to a quadrupole ion trap mass spectrometer, all 96 sample wells could be scanned in 8 min, corresponding to a throughput as high as 720 samples/h (5 s/sample). Even shorter analysis times could, in principle, be obtained with a fast mass spectrometer, such as time-of-flight instrument. It is important to note that unlike the case of flow injection, a useful signal could be observed practically immediately and as long as needed (e.g., MS/MS) before advancing to the next sample.

## EXPERIMENTAL SECTION

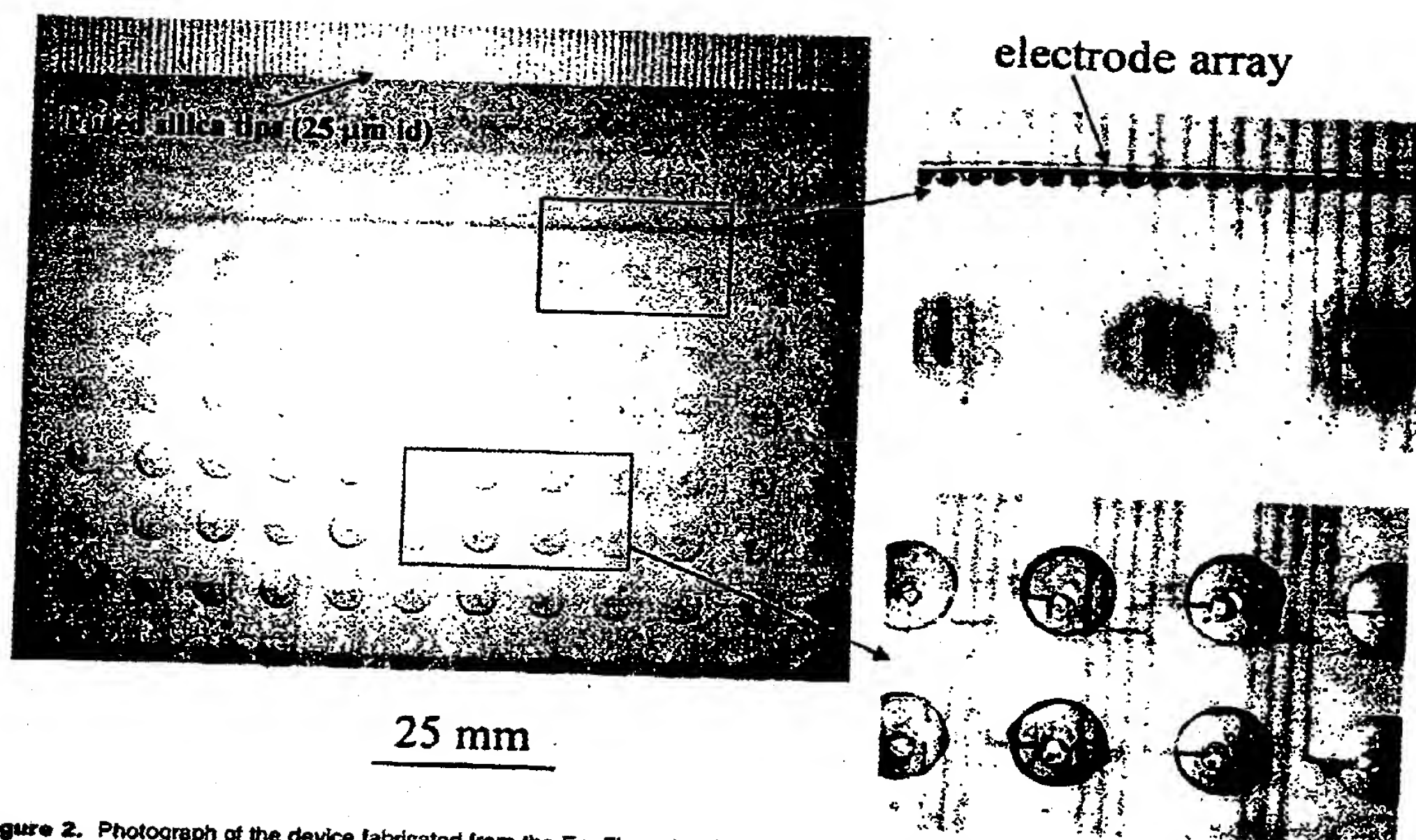
**Fabrication of the Multisprayer Device.** The 96-channel device was fabricated by casting<sup>30,31</sup> from a solvent-resistant polymer resin (EpoFix, EMS, Ft. Washington, PA), as shown in Figure 1. The required patterns of channels and wells (master



**Figure 1.** Fabrication of the 96-ESI channel, 96-well device. (A) Preparation of the silicone rubber negative imprint used for epoxy casting. (B) Flowchart of the device fabrication. Lucite master plates (a, b) were used for preparation of the silicone rubber negative imprints (c, d). After alignment, the final well plate (e) and the bottom plate (f) were cast from the epoxy resin (EpoFix). The device was assembled by bonding the bottom plate to the well plate and gluing the electro-spray tips. The channels are semicircular with 300  $\mu$ m diameter and length of 3–10 cm, depending on the location of the respective well on the plate. Not to scale. See Experimental Section for details.

plates) were first created on rectangular plastic sheets (Lucite S-A-R, Small Parts Inc., Miami Lakes, FL) using a digital drilling machine. Second, the master plates were placed in a plastic box and silicone polymer (Silastic L-RTV silicone rubber kit, Dow Corning Corp., Midland, MI) was cast over the plates. Figure 1A shows the fabrication of the silicone rubber negative with recessed channels of semicircular shape with a diameter of  $\sim 300 \mu$ m. Figure 1B shows the complete flow diagram of the fabrication of the device (only one of the 96 sample wells is depicted). The silicone negative imprints (c and d in Figure 1B) of the Lucite master plates (a and b) were created, as described above. The master

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**Figure 2.** Photograph of the device fabricated from the EpoFix resin with embedded electrodes and attached 96 ESI capillaries (the last row of 12 wells is not visible due to the picture cropping). The expanded sections on the right show the detail of the wells connected to the 300- $\mu$ m-wide semicircular distribution channels (bottom) and of the array of embedded electrodes for sequential connection of the electrospray high voltage.

plate (a) contained 96 channels with starting points distributed in the standard 96-well plate pattern and ending in an array arrangement at the edge of the plate. The master plate (b) contained 96 wells with 5 mm diameter, 5 mm deep, connected to a 0.5-mm-diameter, 0.5-mm-deep hole in the bottom. In the next step, both rubber imprints (c and d) were aligned to form a cavity, which was then filled with the liquid EpoFix resin. Two other polymeric resins were also tested: Acrylic-polyester-based Casolite AP (AIN Plastics, Mt. Vernon, NY) and epoxy-based Araldite (Fluka, Buchs, Switzerland); however, the EpoFix resin exhibited the best mechanical and chemical resistance properties. After hardening, the EpoFix part (e) was recovered and glued together with a bottom plate (f). The bottom plate, also prepared by casting, had 96 embedded electrodes (0.5 mm in diameter, 1.125 mm center-to-center distance). The electrodes were prepared from electrically conductive epoxy (Epo-Tek 415G, Epoxy Technology, Billerica, MA).

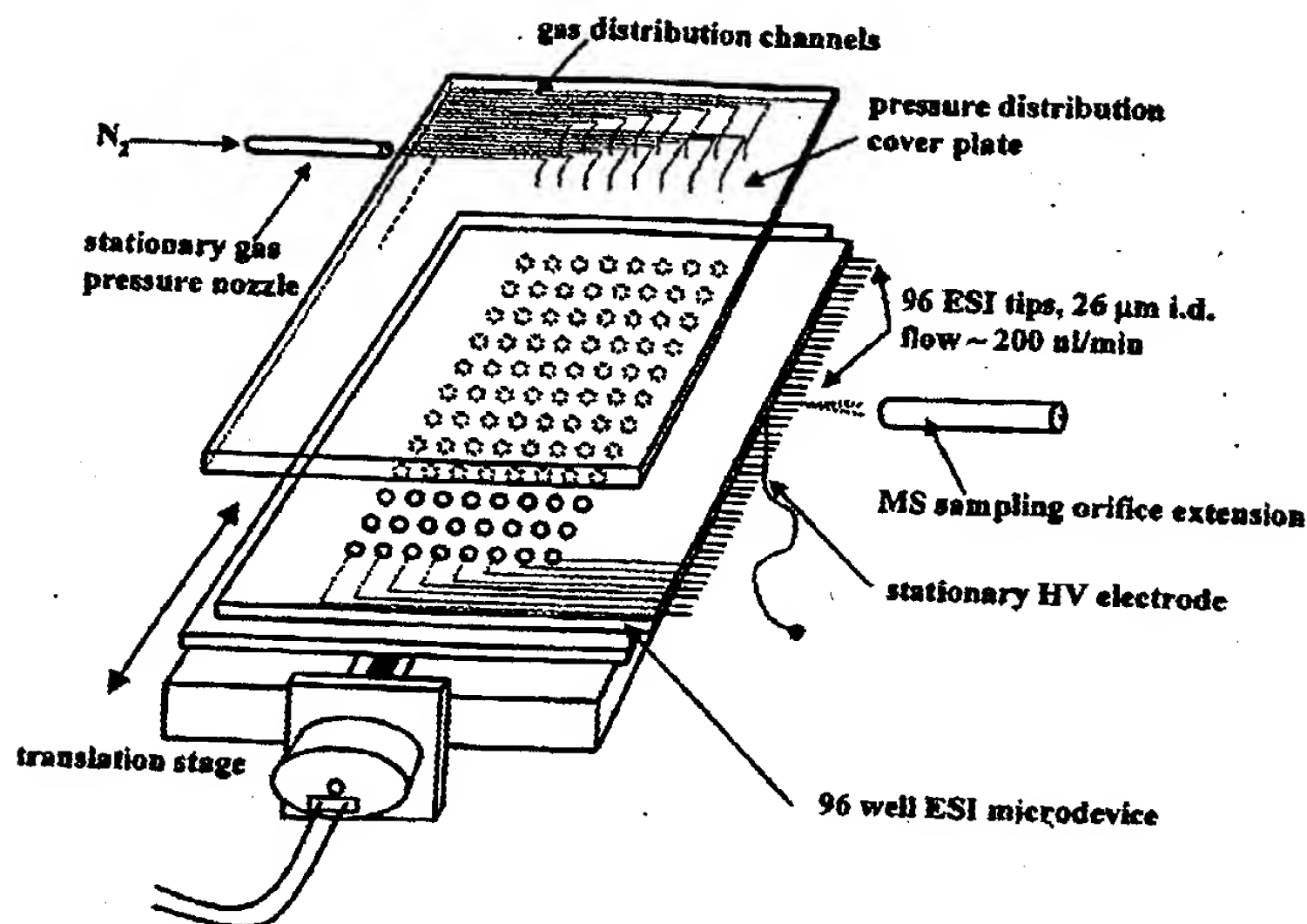
Finally, fused-silica capillaries (2.5 cm in length, 26  $\mu$ m i.d., 140  $\mu$ m o.d.) were inserted into the exits of the channels to a depth of 1.5 cm and glued in place. About 1 mm of the polyimide coating at the capillary tips was removed by heat. This procedure produced a 96-well plate with closed channels and embedded electrodes connecting each well with a separate capillary electrospray tip. The dimensions of the final device, shown in Figure 2, were 16 cm  $\times$  10 cm  $\times$  0.9 cm. The details on the left side of this figure show the individual wells with the channels (bottom) and the array of the electrodes embedded into the channels (top) just prior the attachment of the electrospray tips.

**Mass Spectrometry.** An ion trap mass spectrometer (LCQ, Finnigan MAT, San Jose, CA), operated in the positive ion mode, was used throughout this study. Since the sampling orifice of the instrument was located in a small hemispherical indentation that cannot accommodate the size of the device, an orifice extension was used to overcome the space restriction around the mass spectrometer inlet. The orifice extension was machined from an aluminum rod (2.5 cm long, 8 mm. o.d.) with a 0.35-mm-i.d. channel drilled axially. The extension was connected to the sampling orifice by a 2-cm-long piece of silicone rubber tubing. The signal intensity obtained by electrospraying a standard solution ( $10^{-6}$  M myoglobin, 1% (v/v) acetic acid in 50% methanol/water (v/v)) was  $\sim$ 15% lower with the attached sampling orifice extension, and no further improvement was necessary. The MS data were recorded in the mass-to-charge ranges of 800–1400 (protein samples), 400–1200 (peptide samples), and 300–400 (protease inhibitors).

**System Design and Operation.** The exploded schematic diagram in Figure 3 shows the total system design. During operation, the 96-well/96-ESI tips plate (sample plate) was positioned on a computer-controlled translation stage so that the ESI tips were aligned with the MS sampling orifice extension. The sample plate was then closed by a pressure distribution plate. A thin sheet of silicone rubber with 96 properly positioned holes was placed between the two plates to seal the connection (not shown in Figure 3).

Sequential sample flow through the ESI tips was initiated with the aid of a stationary gas pressure nozzle (200- $\mu$ m-i.d., 1-mm-





**Figure 3.** Exploded view of the total system design. The 96-well plate with individual channels and electrospray tips was positioned on a translation stage in front of the extension of the MS sampling orifice. The electrospray analysis of individual samples was activated by sequential pressurization of the sample wells through the pressure distribution cover plate and connection of the ESI high voltage (HV) through the stationary HV electrode positioned under the ESI device. The silicone rubber sealing gasket placed between the ESI device and the pressure distribution cover plate as well as the aluminum clamping plate was omitted for simplicity. See Experimental Section for details.

o.d. Teflon tube) connected to a nitrogen tank. The nozzle contacted the surface of the pressure distribution cover plate so that channels were individually pressurized during the movement of the translation stage. The pressure distribution cover plate, with well and channel patterns as a mirror image of the sample well plate, was made by the same casting procedure as the sample plate. The stationary high-voltage electrode (1-mm-diameter stainless steel wire) was positioned so that during the movement of the translation stage the high voltage was connected only to the pressurized channel. The high voltage and nitrogen supply were applied during the course of analysis; as the translation stage moved the device to the next position, pressurized gas and high voltage were automatically connected to the respective sample well and channel. An aluminum plate was placed on top of the gas distributor to ensure gastight sealing of all the wells. The linear translation stage (LS3-6-B 10, Del-Tron Precision, Inc., Bethel, CT) was driven by a NEMA 23 step motor controlled by a computer through a motor driver (6006-DB, American Scientific Instrument Corp., Smithtown, NY). A simple computer routine (written in Basic) was used to control the translation stage.

**Chemicals.** Myoglobin, cytochrome *c*, and angiotensins II and III, purchased from Sigma (St. Louis, MO), were each prepared at a concentration of 1 mg/mL and then diluted to the desired concentration with 0.2% (v/v) acetic acid in 50% (v/v) methanol. Fmoc amino acids and H-val-2-chlorotrityl resin were purchased from Anaspec (San Jose, CA). 1-hydroxybenzotriazol (HOBt), 2-(1H-benzotriazole-1,1,3,3-tetramethyluronium) hexafluorophosphate (BBTU), diisopropylethylamine (DIEA), dimethylformamide (DMF), dichloromethane (DCM), potassium cyanide, phenol, ninhydrin, pyridine, and piperidine were obtained from Fluka

(Ronkonkoma, NY). HPLC-grade acetonitrile (ACN) and methanol were also from Fluka. HIV-1 protease was obtained from Pharmacia and Upjohn (Kalamazoo, MI), and pepstatin A and N-acetyl-Thr-Ile-Nle-ψ-[CH<sub>2</sub>N]-Nle-Gln-Arg amine (MVT 101) were from Sigma. The organic compounds 158393, 117027, and 32180 were kindly donated by the Drug Synthesis & Chemistry Branch, Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Hack's balanced salt solution (HBSS) was obtained from Parker-Davis. Mill-Q water (Millipore, Medford, MA) was used throughout.

**Sample Preparation for HIV-1 Protease Inhibition Assay.** An 8-mer peptide substrate (Ser-Gln-Asn-Tyr-Pro-Ile-Val) and a 3-mer peptide internal standard (Glu-Ile-Val) were prepared, following the procedure described in the Anaspec solid-phase synthesis catalog (San Jose, CA). Peptide synthesis was begun from 0.5 mmol of H-val-2-chlorotrityl resin, and coupling was performed by adding 1 mmol of Fmoc amino acid in 1 mmol of HBTU/HOBT, 2 mmol of DIEA. The final peptide was then cleaved from the resin with a mixture of acetic acid/trifluoroacetic acid in dichloromethane and precipitated in ice cold ether. HIV-1 protease inhibition was measured by monitoring the concentration of the enzymatic degradation product—Pro-Ile-Val. The total assay volume was 100 µL, containing 50 µg/mL HIV-1 protease, 1 mM substrate, and a defined amount of inhibitor (pepstatin A or MVT 101) in a MES buffer (100 mM MES, 300 mM KCl, 5 mM EDTA, 4.5% (v/v) DMSO, pH 5.5). The solution was incubated at 37 °C for 90 min and then quenched by addition of 10 µL of TFA. Finally, the solution was spiked with 600 µM Glu-Val-Ile, the internal standard.

Aliquots of sample reaction products of 25–50  $\mu\text{L}$  were taken and desalted on a 96-well  $\text{C}_{18}$  solid-phase extraction (SPE) plate (Varian, Harbor City, CA). The plate was washed with  $3 \times 200 \mu\text{L}$  of methanol followed by  $3 \times 200 \mu\text{L}$  of water. The sample was introduced on the resin and washed extensively ( $4 \times 300 \mu\text{L}$  acidified water (10% (v/v) formic acid)). The sample was then eluted from the SPE resin with  $3 \times 20 \mu\text{L}$  of 1% (v/v) formic acid in 50% (v/v) ACN/ $\text{H}_2\text{O}$ . The eluate solutions were used for direct infusion or were stored in Eppendorf vials at  $-15^\circ\text{C}$  for future analysis.

## RESULTS AND DISCUSSION

The aim of this work was to develop a prototype device for high-throughput infusion ESI-MS with the following features: (1) compatibility with the 96 (or higher)-well plate technology; (2) independent electrospray port attached to each of the sample wells to prevent any sample cross-contamination; (3) potential for fully automated operation.

Although current techniques allow microfabrication of very small devices, the compatibility with current technology is an important issue. At present, 96 (384, 1536)-well plates are used for most of the high-throughput sample processing (enrichment, desalting, etc.).<sup>32–34</sup> Thus, we have designed the device as the final receiving plate in the sample-processing scheme to avoid any need for additional pipetting. Hence, the plate can also be used for sample storage and, if produced from an inexpensive plastic material, can be disposable. These requirements led to a design shown in Figures 2 and 3 where all the sample wells were connected by microchannels to an array of independent electrospray tips on the edge of the device. The size of the device was selected to be compatible with the standard microtiter well plates. Since this size is too large for microfabrication using a standard photolithographic technology with wet chemical etching in glass, we have selected construction by casting with polymeric resins for rapid prototyping.<sup>35,36</sup> The optimum design, suitable for commercial use, could then be produced using injection molding techniques.

In the early stages of this work, we tested several polymeric resins for fabrication of a variety of multichannel devices. Since on-chip fluorescence or absorbance detection was not necessary, the optical properties of the material were not critical; however, the resin could still release impurities (monomers, hardeners, additives, etc.), increasing the MS background and suppressing the analyte signal. During ESI-MS tests with infusion of a myoglobin solution, we found that a device made of Castolite resin generated a strong signal of cluster peaks around  $m/z$  857 that dominated the spectrum and suppressed the protein signal. When the same sample was sprayed from a device made of either Araldite or EpoFix resins, a clean spectrum with strong signal was observed. EpoFix resin was finally selected for fabrication of the 96-well/96-sprayer device due to its superior mechanical properties and ease of use.

Since the diameter of the channels connecting the sample wells with the respective electrospray tips (300  $\mu\text{m}$ ) was much larger than the ESI tip inner diameter (26  $\mu\text{m}$ ), the channel length (3–10 cm) had an insignificant effect on the sample flow rate. Thus, practically all the flow resistance was due to the capillary tip. After application of gas pressure and high voltage, the electrospray stabilized in 1 s, as observed by monitoring the total ion current. At the start of the run, the first of the 96 tips was aligned with the mass spectrometer sampling orifice, with the remaining tips being sequentially positioned automatically at the orifice by means of the fixed step movement of the stage controlled by the computer.

The device was first tested with an aqueous solution of 10  $\mu\text{g}/\text{mL}$  angiotensin II at various pressures (3–40 psi) and voltages (2.5–7 kV), as well as distances between the ESI tip and the MS sampling orifice (1–8 mm). On the basis of signal intensity and stability, 5 psi, 4.5 kV, and 3 mm, respectively, were chosen for all further experiments. Under these conditions, the samples were electrosprayed at a flow rate of  $\sim 200 \text{ nL}/\text{min}$ , i.e., within the optimum range for the capillary electrospray tip.<sup>37</sup> With the motor and the motor driver used, the minimum time required to move from one channel to the next was 1 s; however, much faster stages would be commercially available, if necessary.

**High-Throughput ESI-MS Infusion Analysis.** To demonstrate the high-throughput capability of the system, several sample solutions were alternately deposited in the microtiter wells and then analyzed sequentially and automatically. The spectra of cytochrome c and myoglobin from EIGHT consecutive channels are shown in Figure 4A. Strong signals with well-defined envelopes of the multiply charged protein ions were obtained every 5 s for each consecutive sample. Since fine electrospray capillary tips were used, the electrospray stabilized practically instantly, and no sample cross-contamination was observed.

In a similar experiment shown in Figure 4B, angiotensins II and III were electrosprayed in 8 min from all 96 wells, with singly charged ions of the two peptides being observed. The data demonstrate the validity of the approach to high-throughput infusion analysis where all the samples loaded on the plate can be analyzed in a rapid sequence. Although several channels were blocked during the manual gluing of the device, it can be expected that this would be completely eliminated with improved protocol. Further simplification may also be expected by using a microfabricated array of sprayers instead of individual capillaries<sup>38,39</sup> or microfabrication of the whole device in one piece. It is also worth noting that even higher throughput could be achieved with the use of a time-of-flight, instead of an ion trap, mass spectrometer. Although, a lower limit of detection test was not included in this study, it is reasonable to expect the sensitivity to be equal to that achieved with a single sprayer under the same conditions (tip dimension, sample flow rate, ESI voltage). At a flow rate of 200  $\text{nL}/\text{min}$ , the sample consumption will be minimal even after extended data accumulation (minutes or more) and the unused

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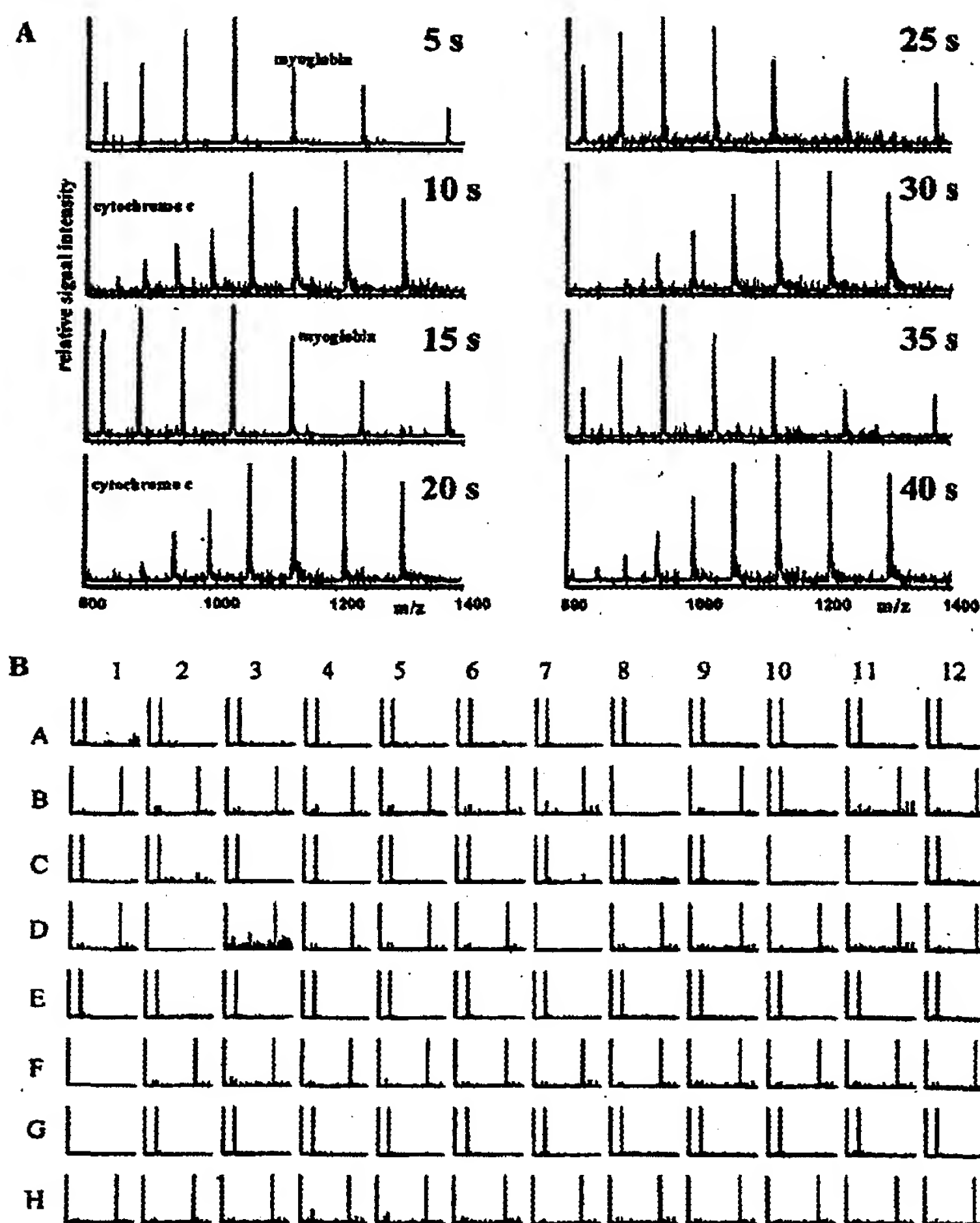
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**Figure 4.** High-throughput ESI-MS analysis using the plastic microwell plate with 96 electrospray tips. (A) cytochrome c and myoglobin solutions (5  $\mu$ L) were alternately loaded into consecutive sample wells, and each well was analyzed every 5 s over a 40-s time period. The concentrations for both proteins were 0.1 mg/mL. (B) angiotensin II and angiotensin III solutions (5  $\mu$ L) were alternately loaded into the sample wells, and all 96 samples were analyzed as in (A). Concentrations of both peptides were 10  $\mu$ g/mL.

samples may be used for additional studies, e.g., enzymatic digestion.

Besides higher throughput, the current device design has additional advantages compared to the ESI-MS analysis performed in the FIA mode. In the latter, the MS signal can be observed for only a limited time, as a result of the fixed injected sample volume and flow rate. In the present system, the signal can be observed almost immediately and as long as desired, allowing a short time to acquire strong signals or a longer time to acquire weak signals of lower concentration samples. Of course, the analysis may be programmed in such a way that the next sample would be

analyzed only after sufficient information, e.g., MS, MS/MS, is obtained. Switching to the next sample is not accompanied by any delays related to the system washing and sample injection. Furthermore, the sample amount consumed can be maintained small ( $\sim 15$  nL or 150 fmol in examples shown in the Figure 4). Moreover, if necessary, practically all the sample deposited in the sample wells can reach the ESI tip and generate useful signal. This would be important with very low concentrated samples or when MS/MS analysis were necessary. In this respect, the device can be viewed as an array of independent nanoelectrosprays allowing extended MS/MS analyses. Since a separate channel and

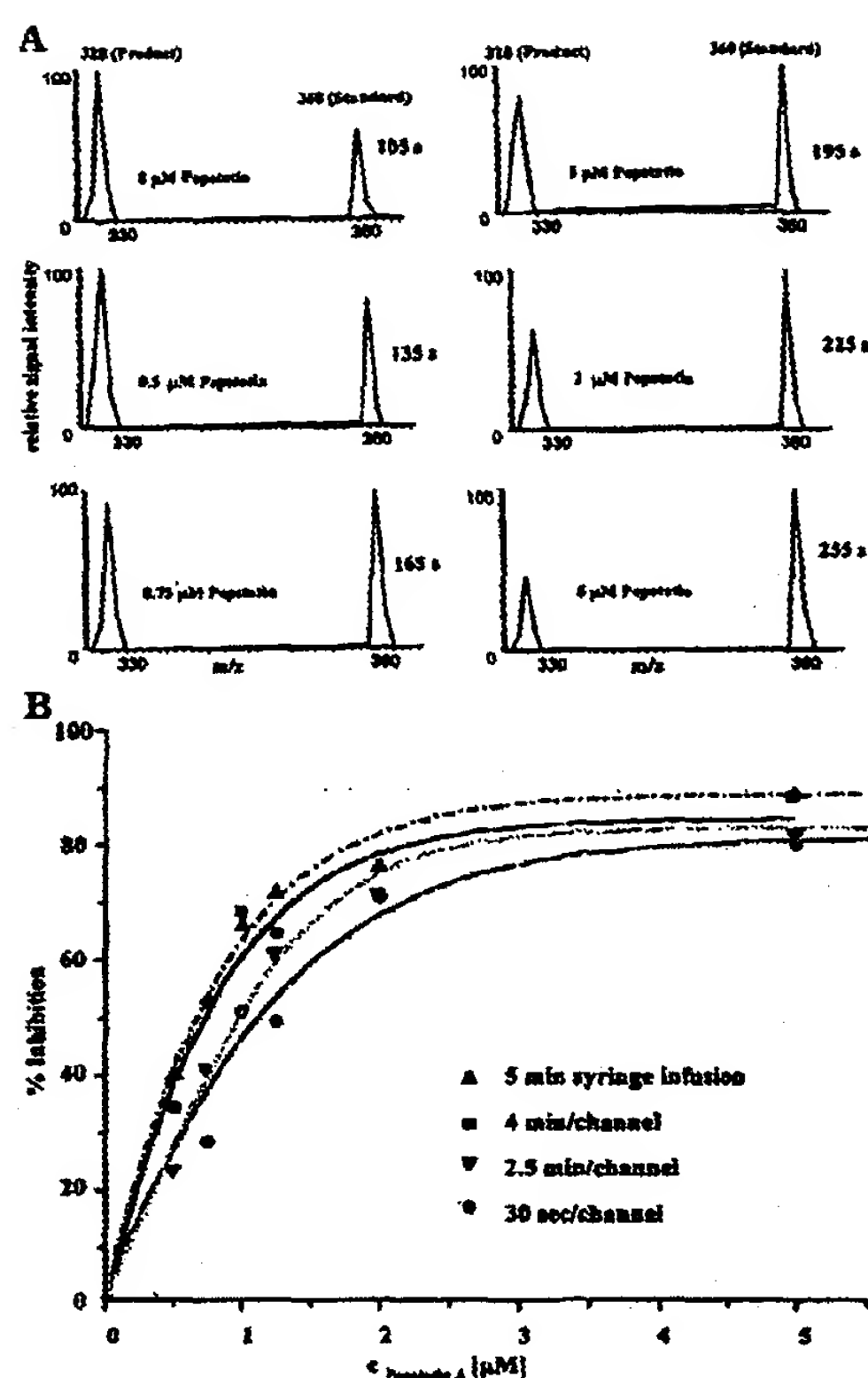
ESI capillary is used for each channel; there is no danger of sample cross-contamination or carryover, which is always a concern in serial flow injection systems.

**HIV-1 Protease Inhibition Assay and  $IC_{50}$  Determination.** As an illustration of the use of the infusion device, we selected to examine the *in vitro* inhibition of HIV-1 protease. The  $IC_{50}$  values (the concentration of an inhibitor necessary to inhibit the enzyme reaction by 50%) were compared with the published data.<sup>40–42</sup> The preparation of a series of samples with increasing concentration of the HIV-1 inhibitor (pepstatin A) was described in detail in the Experimental Section. Prior to ESI-MS analysis, 25- $\mu$ L sample aliquots were desalted on a 96-well  $C_{18}$  SPE plate. The substrate and standard, with no HIV-1 protease added, were also analyzed by direct infusion ESI-MS. No side product formation was observed, except Ser-Gln-Asn-Tyr(*tert*-butyl)-Pro-Ile-Val (MW 875), which was expected from the substrate synthesis. This side product, however, had no influence in the present study since the  $m/z$  value was far removed from the internal standard (MW 359) and the enzymatically formed tripeptide Pro-Ile-Val (MW 327).<sup>43</sup> Figure 5A presents selected ion monitoring (SIM) mass spectra with increasing amounts of inhibitor (pepstatin A), and the corresponding data are plotted in Figure 5B. Inhibition by another peptidomimetic inhibitor *N*-acetyl-Thr-Ile-Nle- $\alpha$ -[CH<sub>2</sub>N]-Nle-Gln-Arg amine, MVT 101 and some other small organic molecules were also studied, and the  $IC_{50}$  obtained are listed in Table 1. The experimental  $IC_{50}$  value of pepstatin A and the  $K_i$  value of MVT 101 were in agreement with those found in the literature<sup>44,45</sup> within the experimental error, typical for this type of analyses (~20%, or more). Since more than 10 MS scans must typically be averaged to obtain a reliable quantitative information, each measurement shown in Figure 5 took ~30 s (10–30 scans averaged). An order of magnitude higher throughput could be obtained with a faster time-of-flight MS instrument. Nevertheless, the model application demonstrates the potential of automated analysis with the present device design.

## CONCLUSIONS

This work presents design principles of a disposable device for high-throughput ESI-MS analysis. The dimensions of the described device were selected to be compatible with the current standard sizes of the microtiter well plates; however, devices with much smaller dimensions can easily be microfabricated, if needed. Since each sample is restricted to its independent fluid path, sample cross-contamination and/or carryover is eliminated. This can significantly simplify the validation of new analytical protocols since the tests for carryover are not necessary. The multichannel device can be viewed as a logical extension of the microtiter well plate technology. All 96 (384, 1536, ...) samples deposited in the microtiter well plate could, in principle, be automatically processed (e.g., incubation, desalting, solid-phase extraction, and affinity

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**Figure 5.** MS determination of HIV-1 protease inhibition using the device. (A) Relative signals of SIM spectra of the product tripeptide (Pro-Ile-Val,  $m/z = 328 \pm 4$ ) and the internal standard (Glu-Ile-Val,  $m/z = 360 \pm 4$ ) after incubation with increasing concentrations of pepstatin A (0–5  $\mu$ M). (B) Plot of the data extracted from (A); the  $IC_{50}$  was determined to be 0.75  $\mu$ M with an RSD of 13%.

**Table 1.  $IC_{50}$  Values of Investigated HIV-1 Protease Inhibitors\***

Inhibitor	inhibitor concn range ( $\mu$ M)	$IC_{50}$ ( $\mu$ M)	
		this work	refs 4 and 45
pepstatin A	0–5	0.75	0.55
MVT 101	0–10	0.65	0.8
compd 117027	0–12.5	9.5	
compd 158393	0–40	6	
compd 32180	0–30	24	

\* Assay conditions: 5  $\mu$ L of 1 mg/mL HIV-1 protease in a 100- $\mu$ L total assay volume, incubation for 90 min at 37  $^{\circ}$ C.

capture) in parallel and finally deposited into the microfabricated device with electrospray tips for rapid sequential MS analysis. By combining parallel off-line SPE sample preparation with the multichannel device-based ESI-MS, sensitive and high-throughput quantitation could be realized (low ng/ $\mu$ L, sample/5 s, RSD 13%).



The device was designed as a disposable counterpart of the standard microtiter well plate and should be a good design strategy in situations where throughput is a key factor, such as compound confirmation and purity estimation of combinatorial libraries, pharmacokinetics studies, and substance aging testing. Arranging ESI tips in a two-dimensional array could further increase the channel density without increasing the size of the device.

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